Eugenol-based O/W Emulsion by Low-energy Emulsification

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Emulsions are systems formed by two immiscible liquids, one of which is dispersed in the other as droplets with a relative stability. These have multiple applications, among them, in the formulation of pharmaceutical and cosmetic products. Its preparation requires generating a large interfacial area, which is usually attained by using the physicochemical formulation know-how on surfactant-oil-water (SOW) systems. Among the applications in the pharmaceutical industry, topical creams, and emulsions for intravenous and for oral administration can be found. Eugenol can be extracted from cloves (Syzygium aromaticum) by various methods, including steam distillation, hydrodistillation and Soxhlet extraction. Furthermore, emulsions based on eugenol can be obtained for a variety of applications, including as topical and oral anesthetic. Nanoemulsions can be formulated with a mixture of non-ionic surfactants Span 20/Tween 80 at an HLB of 11 to 13 and a total surfactant concentration of 4%, using the dilution phase transition method (so-called spontaneous emulsification) to attain stable O/W eugenol-based emulsions. Paraffin oil/eugenol ratio of 4/1 can be used to reach a final emulsion internal oil phase content of 10% with 4% surfactant and 86% aqueous phase. Different polymers are used as viscosifiers, including carboxymethylcellulose. Under these conditions, eugenol-based emulsions with an average droplet size of less than 2 μm can be attained, with topical and oral anesthetic characteristics.

Eugenol emulsion nanoemulsion anesthetic low energy emulsification

1. Nanoemulsion attainment by low-energy methods

Nanoemulsions are non-equilibrated dispersed systems of two immiscible liquids with submicron droplet size[1][2]. Most of the systems formulated to produce nanoemulsions are multicomponent systems[3][4][5]. Therefore, due to the partitioning phenomenon of surfactant species, it is possible to modify the interfacial composition during the emulsification process through the change of water and oil ratios[6][7][8][9]. This is the principle of the emulsification method initially called emulsion inversion point (EIP) described by Marszall[10], used by Lin [11], and improved by Sagitani [12]. The method to obtain nanoemulsions of the oil-in-water (O/W) type consists of adding water to a dispersion formed by the oil and the surfactant mixture until the final submicron emulsion is attained. The importance of microemulsions and/or lamellar liquid crystals phases for the formation of nanoemulsions through a phase-dilution inversion method (also called spontaneous emulsification) has been discussed in previous work[9][13][14]. The generation of these structures such as microemulsions or liquid crystals in surfactant-oil-water (SOW) systems depends on the physicochemical formulation, and particularly, on the surfactant formulation parameter, which can be expressed as HLB or also as SCP or sigma[5][15].

Bullón et al. [3] and Marquez et al. [9] obtained soybean oil in water emulsions stabilized by emulsifiers such as ethoxylated and non-ethoxylated sorbitan esters and lecithin, by using the dilution phase transition method. In both cases, the presence of lamellar liquid crystals was observed. These microstructures make it possible to attain emulsions of submicron droplet sizes after phase transitions during dilution[1][9].

2. Eugenol

The elaboration of micro or nanostructured products in the pharmaceutical field is crucial because they have a large interfacial area and can be used to control active ingredients, such as those present in natural oils[4][16][17]. Eugenol is an essential oil present in cloves *(Syzygium aromaticum).* About 80.7% of clove essential oil is composed of Eugenol. In addition, it contains other compounds such as eugenol acetate (14.8%), ß-caryophyllene (4.1%), and oleanolic triterpene acid (3.2%). The extraction of Eugenol is carried out through different separation methods; among them, the most used are steam distillation, hydrodistillation, and Soxhlet extraction[18][19]. This is used clinically as a local anesthetic and antiseptic, specifically in treating periodontal diseases, due to its antiseptic and anti-inflammatory action[20][21]. It has proven antimicrobial properties against a wide spectrum of bacteria[22][23]. Although its application is common, eugenol can cause caustic injuries or superficial burns when placed directly and in high concentrations in soft tissues. Pure Eugenol at concentrations greater than 10^{-4} mmol/mL (> 600 mg/ml) inhibits cell migration. It modifies the synthesis of prostaglandins, which affects cellular respiration, mitochondrial activity and produces severe changes in the enzymatic activity of the cell membrane[24][25]. For this reason, various vehicles have been used for their application[21].

Eugenol extraction can be performed through three methods[18]:

- Steam distillation: This process consists of co-distillation of the essential oil with water vapor in simple distillation equipment. Thus, the vegetable sample is placed in an inert chamber and subjected to a stream of superheated water vapor, where essential oils that have high boiling points are distilled and then condensed, collected, and separated from the aqueous fraction.

- Hydrodistillation: This is a variant of the simple steam distillation method. The vegetable raw material is loaded into a hydrodistillator to form a compacted bed. The water vapor is injected with enough pressure to overcome the hydraulic resistance of the bed. The steam comes into contact with the clove bed to heat it and release the contained essential oil, which, in turn, evaporates. In this equipment, a trap is placed at the end of the coolant, which separates the oil from the condensed water, which improves and facilitates the extraction of the essential oil.

- Soxhlet extraction: In this method, the solid to be extracted is placed in a cartridge made of filter paper, which is inserted in the center of the chamber. A low boiling point solvent is placed in the balloon and heated to maintain constant reflux. The vapors rise into the condenser, and the condensed liquid falls into the cartridge containing the solid. The solvent fills the chamber and extracts the desired compound from the plant material. Once the cartridge is filled with the solvent, a smaller diameter tube generates a vacuum that drags the solvent with the oil to the distillation balloon.

3. Formulation of eugenol-based O/W emulsions

Formulation scans with nonionic biocompatible surfactants are usually performed to attain O/W nanoemulsions for cosmetic and pharmaceutical applications. An example of such systems is comprised of 4% of the surfactant mixture Span 20 and Tween 80, 8% paraffin oil, 2% eugenol, and 86% aqueous at an HLB formulation parameter (Figure 1). Usually, a minimum droplet size of the emulsion is observed, which is the characteristic behavior of SOW systems at some distance from the optimal formulation[26][27]. In Figure 1 example, at HLB = 12.5, d[3,2] and d(0,9) are similar, indicating that this emulsion is the least polydisperse[3]. This generates greater stability of the emulsions, which is improved by the following factors: 1) a smaller droplet size due to an increase in the interfacial area and an increase in electrostatic and steric repulsion interactions; 2) a less polydisperse droplet size distribution; and 3) the viscosity of the external phase, which can be increased with a viscous agent.

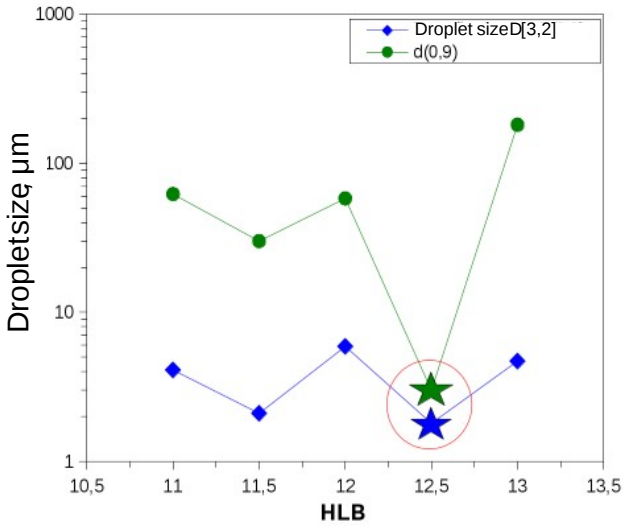

Figure 1. Average droplet size d[3,2] (◆) and d(0,9) (o) of paraffin oil/eugenol/Span 20-Tween 80/Aqueous phase emulsions as a function of HLB. T = 30 C.

Eugenol is a polar oil, making its emulsification difficult due to its low solubilization in SOW systems[9][28][29]. The blend of surfactants Span 20 and Tween 80 has been shown to be suitable for the emulsification of polar oils, such as triglyceride oils[3]. In addition, droplet diameter has to be small, usually less than 2 µm (in Figure 1 case obtained at an HLB = 12.5). Thus, this kind of eugenol encapsulation is more suitable than eugenol oil in its pure state, which can be toxic and generate irritation when applied directly to the skin or in the mouth for local anesthesia, particularly in dentistry[24].

4. Rheological behavior of eugenol emulsions

A suitable formulation of an anesthetic emulsion for a topical application requires [30][31][32][33]:

- High viscosity at low shear for better application.

- Low viscosity at high shear to spread the cream on the skin.

- Wettability and anesthetic effect for several minutes.

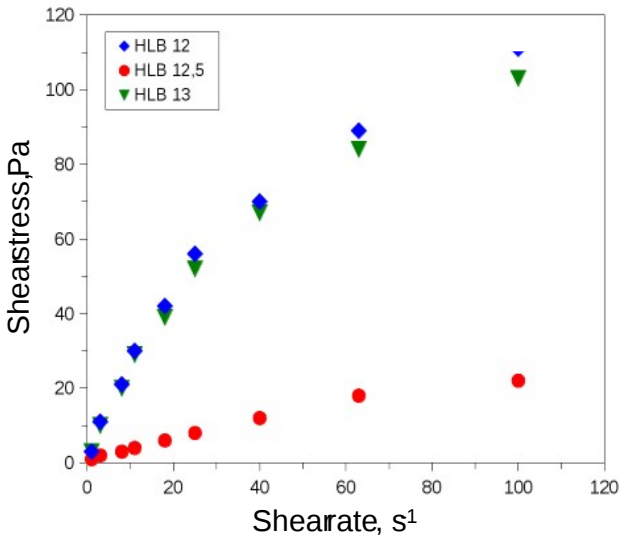

Figure 2. Rheological behavior of emulsions of the paraffin oil/eugenol/Span 20-Tween 80/Aqueous phase system, as a function of HLB. T = 30 C.

The first two characteristics can be improved by using viscosifying polymers, such as carboxymethylcellulose at 3% by weight. Moreover, this polymer can be used in O/W emulsions, modifying the rheological behavior and increasing the stability of the emulsions formed[34]. The third, the anesthetic effect, can be studied through a sensory analysis of the emulsion applied topically on the skin and mouth. This ussually is performed with panel tests.

5. Perspective

Eugenol oil can be extracted from cloves (*Syzygium aromaticum)* by steam distillation and hydrodistillation. Eugenol encapsulation in an O/W submicrosized emulsion, to be used as a topical and oral anesthetic, can be attained by low energy methods by dilution (also called spontaneous emulsification). Emulsion stability, droplet size, and rheological properties are key parameters to vehiculize eugenol for the application as an active ingredient for use as a topical and oral anesthetic.

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